Branching out to gain control: how the pre-TCR is linked to multiple functions

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How is signaling specificity achieved by the pre-TCR during selection of T-cell fate? Like the TCR, this receptor controls many functions, and recent studies define which pathways couple the pre-TCR to the molecular events controlling survival, proliferation, allelic exclusion at the TCRβ locus, and further differentiation.

Development of T cells with an αβ T-cell receptor (TCR) is regulated with great precision by what could be regarded as a developmental forerunner of the TCR, the so-called pre-TCR. This receptor triggers signals for survival, expansion and differentiation in pre-T cells, but the molecular events controlled by the pre-TCR remain ill-defined. How are the pre-TCR signals that lead to survival distinguished from those that lead to proliferation or inhibition of rearrangements at the TCRβ locus? We argue that pre-T cells that have received survival signals from the pre-TCR do not unfold a pre-existing imprinted program of proliferation and differentiation. Rather, pre-TCR-driven signals play an instructive role in promoting development of double-positive (DP) thymocytes, and the pre-TCR signaling pathway required for cessation of TCRβ chain rearrangements is distinct from that which regulates other aspects of αβ T-cell development. Pre-TCR-driven selection of αβ T-cell fate thus emerges as a multi-branched process in which the different cell-fate decisions are orchestrated by different signaling pathways.

Pre-TCR driven selection of αβ T-cell fate

Developing thymocytes follow a strictly regulated developmental program that is initiated after fetal liver or bone-marrow-derived precursor cells enter the thymus. Transition from earlier to later stages depends on signals provided by cytokine receptors (reviewed in Refs 1, 2), as well as by the pre-TCR (reviewed in Refs 1, 3) and the TCR (reviewed in Refs 4, 5). The most immature thymocytes reside within the $CD4^-CD8^-$ double-negative (DN) population (Fig. 1), where TCR gene rearrangements are initiated once CD25 is expressed, and commitment to the T-cell lineage becomes irreversible. TCRβ gene rearrangements are completed at the $CD44^-CD25^+$ stage.

This latter event represents perhaps the most crucial cell-fate-determining event in the development of αβ T cells: only cells that generate a functional TCR β chain mature from the $CD44^-CD25^+$ to the $CD44^-CD25^-$ stage and are selected to develop into αβ T cells[6,7]. This process has been termed 'β selection' and is controlled by the pre-TCR (Ref. 3), a multi-subunit receptor complex comprising the TCR β chain, a pTα chain, and several non-covalently associated polypeptides, referred to as CD3 proteins. Cells that have undergone β selection progress to the $CD4^+CD8^+$ double-positive (DP) stage (reviewed in Refs 1, 3) via $CD8^+$ immature single-positive (ISP) intermediates. Meanwhile, pre-TCR signals terminate rearrangements at the TCRβ locus[8], resulting in allelic exclusion, and TCRα gene transcription and rearrangements are initiated. Although pre-TCR signals might not be absolutely required for TCRβ rearrangements to occur[9], pre-TCR signals are necessary for efficient induction of TCRα gene transcription during the DN to DP transition[10–12]. Following completion of TCRα rearrangements and production of the clonotypic TCRαβ–CD3 complex, DP thymocytes are subjected to a rigorous positive and negative selection process (reviewed in Ref. 5) that forms the repertoire of $CD4^+$ and $CD8^+$ single positive (SP) cells. We shall summarize below the composition and signaling properties of the pre-TCR, and address the different functions it controls in αβ T-cell development. Its instructive role in αβ versus γδ T-cell development was recently addressed elsewhere[13], and will be omitted here owing to space constraints.

Subunit requirements for pre-TCR expression and function

Pre-TCR complexes have a subunit composition[14] that is comparable to αβ TCR complexes (Fig. 2). They contain a TCRβ chain associated with the invariant CD3γ, δ, ε and ζ chains, but the TCRα chain has been replaced by an invariant 33 kDa glycoprotein subunit termed pre-Tα (pTα)[15,16]. pTα has only one immunoglobulin (Ig)-like exodomain loop, compared with two in the TCRα and β chains, prompting speculation that pre-TCRs might contain an additional subunit 'V-preT' (by analogy with the 'V-preB' present in the pre-BCR) that pairs with the V-region of β. Assembly of nascent pre-TCR complexes has not been studied in detail, but the subunit composition of the pre-TCR suggests that its assembly should be similar to that of the αβ TCR, implying that all known subunits are needed. However, unlike the αβ TCR, which requires all of its components (αβγδεζ) for optimal surface expression and function, gene-targeting experiments[17–26] have revealed that pre-TCR expression (and function)

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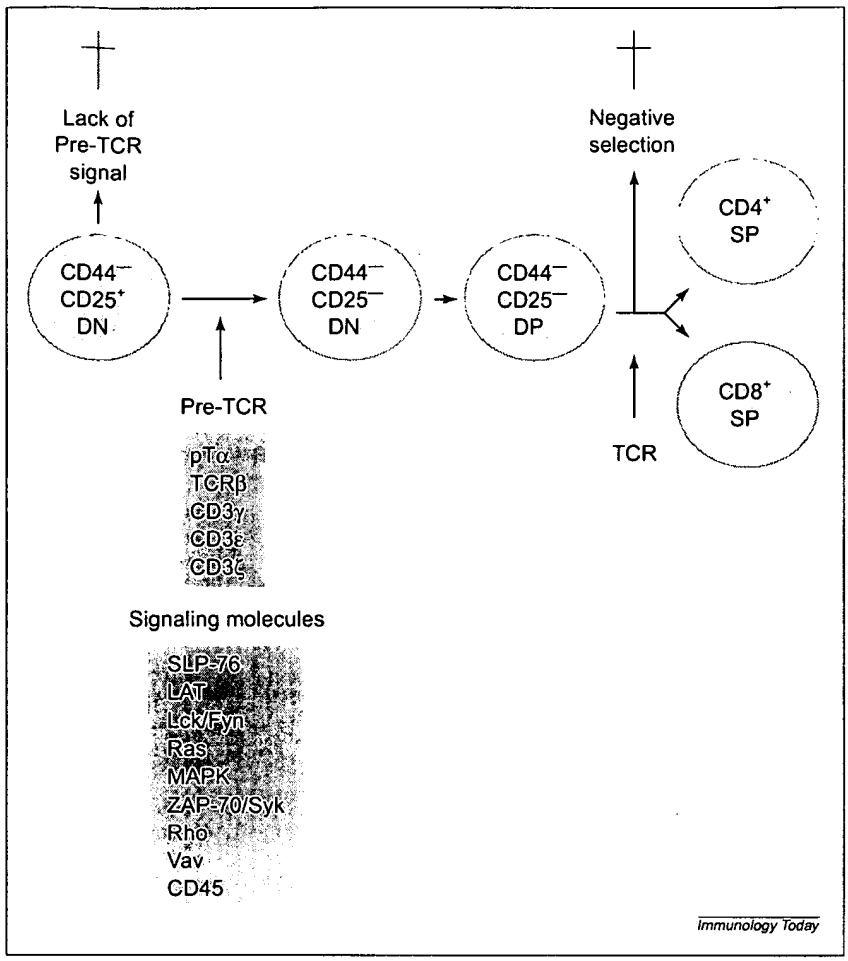

Fig. 1. *Schematic view of the major selection steps in αβ T-cell development. The pre-TCR controls transition from the CD44⁻CD25⁺ to the CD44⁻CD25⁻ stage in the CD4⁻CD8⁻ (double-negative, DN) compartment. At the CD4⁺CD8⁺ (double-positive, DP) stage, TCR signals result either in CD4⁺ or CD8⁺ single-positive (SP) lineage fate, or in cell death (negative selection). The pre-TCR subunits and signaling molecules required for pre-TCR expression and function are depicted in boxes. When the pre-TCR is absent or dysfunctional, DN cells die. Abbreviations: LAT, linker for activation of T cells; TCR, T-cell receptor.*

is less affected by ablation of some of its biochemically defined subunits than αβ TCR expression (see Table 1). Most notably, CD3ζ deficiency only partially impairs pre-TCR function, and CD3δ deficiency has no effect at all. Because pre-TCRs are distinguished from αβ TCRs only by the substitution of pTα for TCRα, these peculiarities of pre-TCR expression are presumably caused by pTα.

Following TCRβ gene rearrangement at the CD44⁻CD25⁺ stage, very low levels of the pre-TCR are expressed on the surface. The surface expression of pre-TCR complexes appears to be low because of two structural features of pTα that limit the extent of pre-TCR assembly: the lack of a V-region domain and lack of its connecting piece (CP), which supports only very weak association with CD3ζ subunits[27]. Consequently, most pTα in DN thymocyte cell lines (and presumably normal DN cells) remains in the endoplasmic reticulum[28].

Initiation of pre-TCR signaling

Although most lymphocyte surface receptor complexes are activated by ligand engagement, for which antibody (Ab) stimulation frequently serves as an effective surrogate, this does not appear to be true for the pre-TCR complex. In fact, Ab engagement of the pre-TCR *in vivo* arrests maturation of thymocytes prior to the DP stage[29]. Moreover, the potential ligand-binding exodomains of pTα and TCRβ are dispensable for pre-TCR function[30,31]. These data suggest that engagement by a specific ligand is not responsible for initiation of pre-TCR signaling *in vivo*. However, it is likely that the pre-TCR needs to be transported to the cell surface of immature thymocytes[32], presumably to meet crucial signaling components.

How then is signaling initiated? It appears that the pre-TCR has a unique capability to transduce β-selection signals even when expressed at low levels and when not engaged by a surface ligand. There are several hypotheses to explain this phenomenon, all of which have in common the concept that pre-TCRs are constitutively active signaling complexes, and that this constitutive signaling ability is conferred upon the pre-TCR by some unique property of pTα (Refs 30, 32). If so, then that 'property' of pTα probably maps to its CP or its transmembrane domain, because the Ig loop and cytoplasmic tail are dispensable for function, at least when overexpressed[33]. It is unclear how pTα might confer constitutive signaling ability upon the pre-TCR, but it has been suggested that pTα does so by directly targeting pre-TCR complexes into lipid microdomains, which are enriched in glycolipids and signaling molecules involved in T-cell activation (also known as rafts, GEMs, DIMs) (reviewed in Ref. 34). In agreement with this suggestion, a recent report[35] suggests that pTα is palmitoylated, and that its palmitoyl moiety targets the pre-TCR to lipid microdomains. Further investigation is required to resolve whether this is indeed the basis for ligand-independent signaling of the pre-TCR.

Proteins involved in pre-TCR signaling

Regardless of how pre-TCR signaling is initiated, its CD3 components endow it with its molecular sensor properties. Insights into the signaling components involved have been obtained largely through natural or engineered mutations, and through functional inactivation studies in fetal thymus organ cultures (Tables 1, 2).

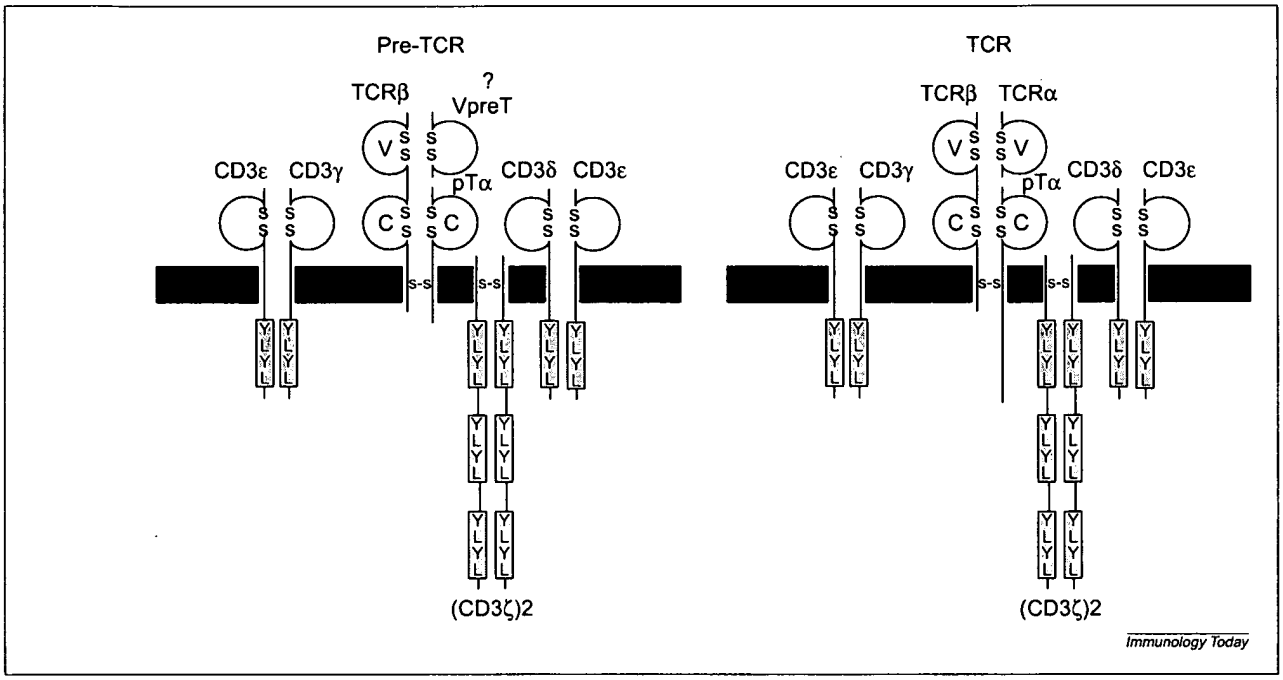

Fig. 2. *Subunit composition of the pre-TCR and the TCR. Pre-TCRs are distinguished from αβ TCRs only by the substitution of pTα for TCRα. The pTα chain has only one immunoglobulin-like exodomain loop, and the VpreT-loop depicted here is based solely on speculation: by analogy with the VpreB-loop in the pre-BCR, such a component might exist. Another difference between the pre-TCR and the TCR is that CD3δ is difficult to detect and not required for pre-TCR function, whereas in the αβTCR, it is required for both assembly and function. The 'YLYL' motifs shared by all CD3 chains represent the immunoreceptor tyrosine-based activation motifs (ITAMs) that, after phosphorylation, couple the pre-TCR and the TCR to the adaptors and kinases critical for signaling. Abbreviation: TCR, T-cell receptor.*

The CD3 components that form part of the pre-TCR (and of the mature TCR, for that matter) contain one or several immunoreceptor tyrosine-based activation motifs (ITAMs) within their cytoplasmic domain. As in the TCR, these ITAMs are phosphorylated by activated Src-family protein tyrosine kinases (PTKs) which, together with their regulatory enzymes, play critical roles in pre-TCR signaling[33,36–43]. Phosphorylation of the ITAMs creates docking sites for SH2-domain-containing Syk-family PTKs, ZAP-70 and Syk, both of which contribute to pre-TCR function[44,45].

More distal events in pre-TCR signaling are regulated by adaptors, exchange factors and GTPases, all of which are involved in mature TCR signaling. Indeed, the adaptor proteins SLP-76 and LAT (linker for activation of T cells) are crucial for pre-TCR signaling[46–48], as are the GTPases p21ras (Ref. 49) and Rho (Refs 50, 51), and (to a lesser extent) the guanine nucleotide exchange factor Vav (Refs 52–55). In addition, the Ras–Raf–MAPK–ERK cascade has been implicated in pre-TCR signaling, in that introduction of constitutively active components of this pathway restores development of pre-TCR deficient thymocytes to the DP stage[56,57]. Finally, a novel reporter plasmid system for detecting ERK activation within the developing thymocytes showed that formation of the pre-TCR leads to activation of this serine/threonine kinase[58]. ERK's substrate, the transcription factor Elk-1, can also be activated by the JNK pathway, but inactivation of key components in this pathway does not affect β selection[59–61].

How might Elk-1 and other transcription factors regulate β selection? This is still an area of much speculation because the nuclear targets of the signaling pathways triggered by the pre-TCR are largely unknown. Nevertheless, several transcription factors have been implicated in β selection (Table 2), although their putative targets at this developmental stage remain to be defined. The E family of bHLH (basic helix-loop-helix) transcription factors (E2-2, HEB and E2A) plays key roles in multiple developmental pathways, and affects the DN to DP transition: mice lacking HEB (Ref. 62) have a decrease in DP and increase in ISP thymocytes. The Ikaros family of hematopoietic-specific transcription factors are essential for T-cell lineage commitment, and act beyond the DN stage: pre-T cells lacking Ikaros can perform β selection without the pre-TCR (Ref. 63). Ablation of both the high mobility group (HMG) box transcription factors Tcf-1 and Lef-1 prevents thymocyte development beyond the ISP stage[10]. The early growth response gene family member 1 (Egr-1) is upregulated in β-selected cells, and its enforced expression relieves the developmental arrest in pre-TCR-deficient thymocytes[64]. Egr family members regulate expression of many gene targets of β-selection signals, including TCRα, pTα and the Rag genes (M. Carleton *et al.*, unpublished). Investigations are currently under way to discover which other aspects of β selection are controlled by this family of transcription factors.

In conclusion, many of the membrane-proximal signaling effectors activated by the mature TCR are used in pre-TCR signaling. How

Table 1. Mutations that have provided insights in the regulation of αβ T-cell lineage differentiation[a]

Deficiency	Phenotype	Refs
pTα	Block at $CD44^-CD25^+$ stage	8, 17
	Rescue with activated Lck, tailless pTα anti-CD3	
TCRβ	Block at $CD44^-CD25^+$ stage	25
	Rescue with anti-CD3	75
TCRα	Block at DP stage	25
CD3γ	Block at $CD44^-CD25^+$ stage	20
	Rescue with activated Lck, anti-CD3, loss of p53	38
CD3δ	Block at DP stage	14, 18
CD3ε	Block at $CD44^-CD25^+$ stage	19, 26
CD3ζ	Partial block at DN to DP transition	21–24
Lck	Partial block at DN to DP transition	36
	Partial rescue ($Rag^{-/-} \times Lck^{-/-}$) with anti-CD3	76
Fyn	No β selection defect	39, 40
Lck/Fyn	Severe block at $CD44^-CD25^+$ stage	39, 40
Csk	Bypasses need for pre-TCR and TCR	41
CD45	Partial block at DN to DP transition	42, 43
ZAP-70/Syk	Block at $CD44^-CD25^+$	44, 45
SLP-76	Block at $CD44^-CD25^+$	46, 48
	No rescue with anti-CD3	
LAT	Block at $CD44^-CD25^+$	47
Vav	Partial defect in DN to DP transition	52–55
Rho	Block at DN to DP transition	50, 51
Rag	Block at $CD44^-CD25^+$ stage	77, 78
	Rescue with activated Lck,	37
	with activated Ras, Raf,	49, 56, 57
	with DN-FADD,	69
	anti-CD3	75, 79, 80

[a]Abbreviations: TCR, T-cell receptor; DP, double-positive ($CD4^+CD8^+$); DN, double-negative ($CD4^-CD8^-$); DN-FADD, dominant-negative Fas-associated death domain; LAT, linker for activation of T cells.

then are these shared signaling events translated into distinct biological functions? Whereas both the mature TCR and the pre-TCR can trigger anti-apoptotic events and proliferative signals, the pre-TCR triggers several additional outcomes, including termination of TCRβ locus rearrangements and induction of CD4 and CD8 expression. Are these distinct cellular outcomes the consequence of a common signaling machinery defined by different cellular contexts? For other pathways, notably Ca^{2+} signaling[65], this certainly can be the case. The specificity of Ca^{2+} signaling is determined in part by selective use of downstream transcriptional targets that are differentially expressed in certain cell types. Furthermore, differences in concentrations of crucial cytoplasmic and nuclear mediators between cell types might dictate the outcome of generic signals. Determining to what extent the differences in outcome between pre-TCR and TCR signaling might be shaped by cellular context remains a task for the future. Possibly, some answers will be obtained in the not too distant future, through representational difference analysis and gene expression arrays. Moreover, the pre-TCR might produce different biological outcomes by coupling to cytoplasmic mediators distinct from those used by the TCR; identification of such mediators might be a fruitful area of research.

How to traverse the β selection checkpoint with one sensor

Why must the signaling network used by the pre-TCR be so elaborate? The answer probably lies in the many different functions it controls. First, DN cells lacking a pre-TCR have a survival defect, implicating the pre-TCR in providing crucial anti-apoptotic signals. Second, transition from the DN to the DP stage is accompanied by prolific expansion, and signaling through the pre-TCR is essential for entering this burst of cell proliferation. Third, once functional rearrangements of one TCRβ allele have occurred and the pre-TCR is formed, pre-TCR signals terminate rearrangements at the remaining TCRβ allele, thus ensuring allelic exclusion. Finally, pre-TCR signals control further maturation to the DP stage, regulating numerous genes, including those required for induction of CD4 and CD8 expression, for downmodulation of pTα gene expression, and those involved in transactivation of the TCRα locus prior to its rearrangement. Here, we consider how signal specification can be achieved by the pre-TCR (Fig. 3), searching for clues in gene ablation and gene complementation studies.

The notion that rescue from cell death is not the only task of the pre-TCR has become clear as the molecular mediators involved in its pro-survival role are explored. The survival defect of pre-TCR-deficient $CD25^+$ DN cells, or $CD25^+$ DN cells whose pre-TCR signaling has been attenuated (due to loss of Rho function) is manifested only in the presence of the tumor suppressor gene p53 (Refs 66, 67). Importantly, expression of transgenic Bcl2 does not rescue survival and differentiation of CD3γ-deficient mice[38], consistent with the earlier report[68] that overexpression of Bcl2 can rescue the survival defect in interleukin-7-receptor-deficient mice, but not that of Rag1-deficient thymocytes lacking the pre-TCR.

Table 2. Transcriptional control of the DN to DP transition[a]

Transcription factors implicated in β selection	Observation	Refs
bHLH (E family)	HEB deficiency results in an increase in ISPs and decrease in DP	62
Ikaros family	Without Ikaros function, transition to the DP stage becomes pre-TCR-independent	63
HMG family	Mice lacking both Lef-1 and TCF-1 fail to transit beyond the ISP stage	10
Egr family	Enforced Egr-1 expression allows transition to the ISP stage in Rag-deficient mice	64

[a]Abbreviations: bHLH, basic helix-loop-helix; DN, double-negative ($CD4^-CD8^-$); DP, double-positive ($CD4^+CD8^+$); Egr-1, early growth response gene family member 1; HMG, high mobility group; ISP, immature single-positive ($CD8^+CD3$); TCR, T-cell receptor.

The studies in p53-deficient mice are important not only because they place inactivation of p53 directly downstream from pre-TCR signaling, but also because they reveal what p53-deficiency cannot achieve. Genetic removal of p53, in isolation, fails to rescue thymic cellularity and only minimally rescues differentiation of the pre-TCR-deficient thymocytes to the DP stage[66]. Moreover, in mice with a crippled pre-TCR, p53-deficiency restores expansion and differentiation to the DP stage only after a protracted time period, despite full rescue of pre-T-cell survival[38]. Defective pre-TCR driven proliferation and differentiation signals are the most likely explanations for these findings, underscoring the notion that the signal for survival bifurcates upstream from those regulating proliferation and further maturation. In addition, the signals for maturation and proliferation might be separable, because expression of dominant-negative Fas-associated death domain (DN-FADD) in pre-T cells that lack a pre-TCR rescues survival and differentiation but not proliferation[69]. In agreement with these findings, pre-T cells that express DN-FADD but lack a pre-TCR are resistant to the proliferative response that can be induced by CD3 ligation[69]. These findings suggest not only that inactivation of FADD can obviate the need for pre-TCR survival signals, but also that FADD is required for pre-TCR-driven proliferation signals and not for maturation signals.

Which other signaling effectors might be positioned at key branch points? As mentioned above, activation of the Src-family PTK Lck appears necessary and sufficient to provide all pre-TCR driven β-selection events, because expression of an activated Lck transgene in numerous mouse strains with natural or engineered pre-TCR defects (Table 1) can fully restore the differentiation program normally orchestrated by the pre-TCR. However, Lck's ability to trigger proliferation and differentiation can be distinguished from its ability to drive allelic exclusion by its dependence on Rho (Ref. 70), suggesting a branching model for pre-TCR signaling downstream of Lck. All β-selection events, including allelic exclusion, are dependent on SLP-76 (Refs 46, 48, 71), probably due to the fact that SLP-76 is a critical scaffold protein for several adaptors, regulators of Ras signaling, and phospholipase Cγ1 (PLCγ1)[72,73]. Further important clues about bifurcation of pre-TCR signals were provided last year, when two groups reported on the role of the Ras–Raf–MAPK pathway in β selection[56,57]. In the absence of a pre-TCR, active Ras-V12 (Ref. 57) and active Raf-CAAX (Ref. 56) expressed in Rag-deficient thymocytes trigger survival and proliferation, and transition to the DP stage. However, in normal mice, they

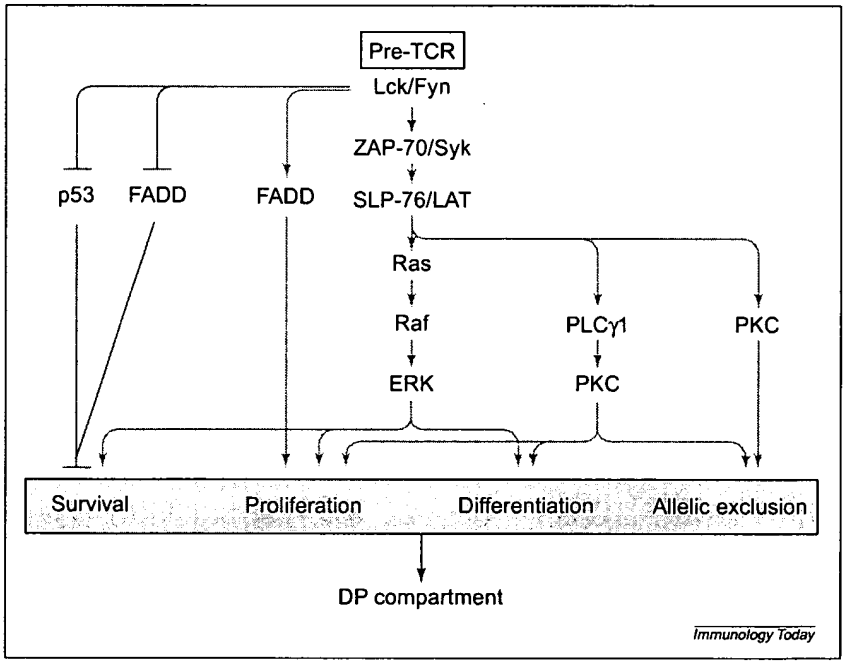

Fig. 3. *Proposed model for signal specification by the pre-TCR. Gene ablation and gene complementation studies have implicated the signaling mediators depicted here. Other components involved in signal specification are likely to be defined, but the present-day knowledge of the mediators required for pre-TCR driven selection already support this multi-branched, instructive model. Activation of Lck is necessary and sufficient for all pre-TCR-driven selection events. Signals for survival bifurcate upstream of those for proliferation and differentiation, and the Ras–Raf–ERK pathway regulates survival, proliferation and differentiation. By contrast, signals for TCRβ allelic exclusion bifurcate upstream of Ras and downstream of SLP-76/LAT, and require PKC activation. Abbreviations: DP, double-positive ($CD4^+CD8^+$), FADD, Fas-associated death domain; LAT, linker for activation of T cells; PLCγ1, phospholipase Cγ1; PKC, protein kinase C.*

fail to induce termination of TCRβ chain rearrangements. These studies indicate that TCRβ allelic exclusion is not a by-product of differentiation, but actually requires specific signals that bifurcate upstream of the Ras–Raf–MAPK cascade. Together with the above cited role of Lck and SLP-76 in allelic exclusion, these findings place the bifurcation of signals for allelic exclusion upstream of Ras activation, and downstream of Lck/ZAP-70-mediated phosphorylation of SLP-76.

Because one of the roles of SLP-76 is triggering protein kinase C (PKC) activation by PLCγ1, PKC appears to be another likely point of signal bifurcation. The PKC-θ isoform has been implicated in TCR signaling in mature T cells, but does not have a specific role in β selection: mice lacking PKC-θ have a severe defect in mature T-cell activation, but normal thymocyte development[74]. Might other isoforms of PKC then be involved? Indeed, that does appear to be the case: PKC is activated upon formation of the pre-TCR complex (A.M. Michie *et al.*, unpublished), and this activation results in proliferation and maturation of DN thymocytes. Moreover, an activated form of PKC-α was shown to enforce allelic exclusion at the TCRβ locus (A.M. Michie *et al.*, unpublished). PKC signals are thus critical mediators of pre-TCR signal specification, and can be positioned at the branch point where signals leading to proliferation and differentiation diverge from those enforcing allelic exclusion. These findings illustrate that the pre-TCR and TCR can couple to different PKC isoforms. Thus, although the pre-TCR and TCR share many proximal signaling events, the different biological outcomes of triggering these receptors might relate in part to their coupling to different mediators. Identification of additional pre-TCR-binding mediators will be critical in understanding its specific biological role, as will be the identification of specific target genes.

Concluding remarks

One of the most fascinating challenges in T-cell biology is to define how signaling specificity is directed by TCRs and pre-TCRs during selection of T-cell fate, be it in mature T cells or in their precursors. From data such as those reviewed above, we arrive at the view that the pre-TCR-driven transition from the DN to the DP compartment is a multi-branched program, in which the pathways leading to developmental progression and expansion can be distinguished from those resulting in survival and termination of TCRβ chain rearrangements. The inevitable conclusion is that the pre-TCR couples to multiple functions through different pathways, in ways yet to be fully defined. Of course, this situation is no different than that of the mature TCR, whose differential signaling capabilities have been studied for many years. Because both receptors are associated with an elaborate signaling network, subtle distinctions in initial activation or recruitment of proximal signaling effectors can be propagated into different signaling outcomes, controlling specific cell fate decisions and differentiation steps.

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References

1 Haks, M.C. *et al.* (1999) Cell-fate decisions in early T cell development: regulation by cytokine receptors and the pre-TCR. *Sem. Immunol.* 11, 23–37
2 Di Santo, J.P. and Rodewald, H-R. (1998) ***In vivo*** roles of receptor tyrosine kinases and cytokine receptors in early thymocyte development. *Curr. Opin. Immunol.* 10, 196–207
3 Von Boehmer, H. *et al.* (1999) Pleiotropic changes controlled by the pre-T cell receptor. *Curr. Opin. Immunol.* 11, 135–142
4 Bevan, M.J. (1997) In thymic selection, peptide diversity gives and takes away. *Immunity* 7, 175–178
5 Amsen, D. and Kruisbeek, A.M. (1998) Thymocyte selection: not by TCR alone. *Immunol. Rev.* 165, 209–229
6 Shinkai, Y. *et al.* (1993) Restoration of T cell development in RAG-2-deficient mice by functional TCR transgenes. *Science* 259, 822–825
7 Dudley, E.C. *et al.* (1994) T cell receptor β chain gene rearrangement and selection during thymocyte development in adult mice. *Immunity* 1, 83–93
8 Aifantis, I. *et al.* (1997) Essential role of the pre-T cell receptor in allelic exclusion of the T cell receptor β locus. *Immunity* 7, 601–607
9 Mancini, S. *et al.* (1999) TCR α-chain repertoire in pTα-deficient mice is diverse and developmentally regulated: implications for pre-TCR functions and TCRA gene rearrangement. *J. Immunol.* 163, 6053–6059
10 Okamura, R.M. *et al.* (1998) Redundant regulation of T cell differentiation and TCRα gene expression by the transcription factors LEF-1 and TCF-1. *Immunity* 8, 11–20
11 Hernández-Munain, C. *et al.* (1999) A developmental switch from TCRδ enhancer to TCRα enhancer function during thymocyte maturation. *Immunity* 10, 723–733
12 Koyasu, S. *et al.* (1997) Pre-TCR signaling components trigger transcriptional activation of a rearranged TCRα gene locus and silencing of the pre-TCRα locus: implications for intrathymic differentiation. *Int. Immunol.* 9, 1475–1480
13 Aifantis, I. *et al.* (1998) On the role of the pre-T cell receptor in αβ versus γδ T lineage commitment. *Immunity* 9, 649–655
14 Berger, M.A. *et al.* (1997) Subunit composition of pre-T cell receptor complexes expressed by primary thymocytes: CD3δ is physically associated but not functionally required. *J. Exp. Med.* 186, 1461–1467
15 Groettrup, M. *et al.* (1993) A novel disulfide-linked heterodimer on pre-T cells consists of the T cell receptor β chain and a 33 kd glycoprotein. *Cell* 75, 283–294
16 Saint-Ruf, C. *et al.* (1994) Analysis and expression of a cloned pre-T cell receptor gene. *Science* 266, 1208–1212

17 Fehling, H.J. *et al.* (1995) Crucial role of the pre-T-cell receptor α gene in development of αβ but not γδ T cells. *Nature* 375, 795–798
18 Dave, V.P. *et al.* (1997) CD3δ deficiency arrests development of the αβ but not the γδ T cell lineage. *EMBO J.* 16, 1360–1370
19 Malissen, M. *et al.* (1995) Altered T cell development in mice with a targeted mutation of the CD3-ε gene. *EMBO J.* 14, 4641–4653
20 Haks, M.C. *et al.* (1998) The CD3γ chain is essential for development of both the TCRαβ and TCRγδ lineages. *EMBO J.* 17, 1871–1882
21 Love, P.E. *et al.* (1993) T cell development in mice that lack the ζ chain of the T cell antigen receptor complex. *Science* 261, 918–921
22 Malissen, M. *et al.* (1993) T cell development in mice lacking the CD3-ζ/η gene. *EMBO J.* 12, 4347–4355
23 Ohno, H. *et al.* (1993) Developmental and functional impairment of T cells in mice lacking CD3ζ chains. *EMBO J.* 12, 4357–4366
24 Liu, C-P. *et al.* (1993) Abnormal T cell development in CD3-$\zeta^{-/-}$ mutant mice and identification of a novel T cell population in the intestine. *EMBO J.* 12, 4863–4875
25 Mombaerts, P. *et al.* (1992) Mutations in T-cell antigen receptor genes α and β block thymocyte development at different stages. *Nature* 360, 225–231
26 DeJarnette, J.B. *et al.* (1998) Specific requirement for CD3α in T cell development. *Proc. Natl. Acad. Sci. U. S. A.* 95, 14909–14914
27 Trop, S. *et al.* (1999) The connecting peptide domain of pTα dictates weak association of the pre-T cell receptor with the TCR ζ subunit. *Eur. J. Immunol.* 29, 2187–2196
28 Trop, S. *et al.* (2000) Competitive displacement of pTα by T cell receptor (TCR)-α during TCR assembly prevents surface coexpression of pre-TCR and αβ TCR. *J. Immunol.* 165, 5566–5572
29 Takahama, Y. *et al.* (1995) Regulation of early T cell development by the engagement of TCR-β complex expressed on fetal thymocytes from TCR-β-transgenic *scid* mice. *J. Immunol.* 154, 5862–5869
30 Irving, B.A. *et al.* (1998) Thymocyte development in the absence of pre-T cell receptor extracellular immunoglobulin domains. *Science* 280, 905–908
31 Jacobs, H. *et al.* (1996) Oncogenic potential of a pre-T cell receptor lacking the TCRβ variable domain. *Oncogene* 12, 2089–2099
32 O'Shea, C.C. *et al.* (1997) Exit of the pre-TCR from the ER/*cis*-Golgi is necessary for signaling differentiation, proliferation, and allelic exclusion in immature thymocytes. *Immunity* 7, 591–599
33 Fehling, H.J. *et al.* (1997) Restoration of thymopoiesis in pTα$^{-/-}$ mice by anti-CD3ε antibody treatment or with transgenes encoding activated Lck or tailless pTα. *Immunity* 6, 703–714
34 Dustin, M.L. and Cooper, J.A. (2000) The immunological synapse and the actin cytoskeleton: molecular hardware for T cell signaling. *Nat. Immunol.* 1, 23–29
35 Saint-Ruf, C. *et al.* (2000) Different initiation of pre-TCR and γδTCR signalling. *Nature* 406, 524–527
36 Molina, T.J. *et al.* (1992) Profound block in thymocyte development in mice lacking p56^{lck}. *Nature* 357, 161–164
37 Mombaerts, P. *et al.* (1994) An activated *lck* transgene promotes thymocyte development in *RAG-1* mutant mice. *Immunity* 1, 261–267
38 Haks, M.C. *et al.* (1999) Pre-TCR signaling and inactivation of p53 induces crucial cell survival pathways in pre-T cells. *Immunity* 11, 91–101
39 Van Oers, N.S.C. *et al.* (1996) αβ T cell development is abolished in mice lacking both Lck and Fyn protein tyrosine kinases. *Immunity* 5, 429–436
40 Groves, T. *et al.* (1996) Fyn can partially substitute for Lck in T lymphocyte development. *Immunity* 5, 417–428
41 Schmedt, C. *et al.* (1998) Csk controls antigen receptor-mediated development and selection of T-lineage cells. *Nature* 394, 901–904
42 Kishihara, K. *et al.* (1993) Normal B lymphocyte development but impaired T cell maturation in CD45-exon6 protein tyrosine phosphatase-deficient mice. *Cell* 74, 143–156
43 Byth, K.F. *et al.* (1996) CD45-null transgenic mice reveal a positive regulatory role for CD45 in early thymocyte development, in the selection of CD4$^+$CD8$^+$ thymocytes, and in B cell maturation. *J. Exp. Med.* 183, 1707–1718
44 Cheng, A.M. *et al.* (1997) The Syk and ZAP-70 SH2-containing tyrosine kinases are implicated in pre-T cell receptor signaling. *Proc. Natl. Acad. Sci. U. S. A.* 94, 9797–9801
45 Sugawara, T. *et al.* (1998) An improved retroviral gene transfer technique demonstrates inhibition of CD4$^-$CD8$^-$ thymocyte development by kinase-inactive ZAP-70. *J. Immunol.* 161, 2888–2894
46 Clements, J.L. *et al.* (1998) Requirement for the leukocyte-specific adapter protein SLP-76 for normal T cell development. *Science* 281, 416–419
47 Zhang, W. *et al.* (1999) Essential role of LAT in T cell development. *Immunity* 10, 323–332
48 Pivniouk, V. *et al.* (1998) Impaired viability and profound block in thymocyte development in mice lacking the adaptor protein SLP-76. *Cell* 94, 229–238
49 Swat, W. *et al.* (1996) Activated Ras signals differentiation and expansion of CD4$^+$8$^+$ thymocytes. *Proc. Natl. Acad. Sci. U. S. A.* 93, 4683–4687
50 Galandrini, R. *et al.* (1997) Different functions of the GTPase Rho in prothymocytes and late pre-T cells. *Immunity* 7, 163–174
51 Costello, P.S. *et al.* (2000) The GTPase Rho controls a p53-dependent survival checkpoint during thymopoiesis. *J. Exp. Med.* 192, 77–85
52 Fischer, K-D. *et al.* (1995) Defective T-cell receptor signalling and positive selection of Vav-deficient CD4$^+$CD8$^+$ thymocytes. *Nature* 374, 474–477
53 Turner, M. *et al.* (1997) A requirement for the Rho-family GTP exchange factor Vav in positive and negative selection of thymocytes. *Immunity* 7, 451–460
54 Tarakhovsky, A. *et al.* (1995) Defective antigen receptor-mediated proliferation of B and T cells in the absence of Vav. *Nature* 374, 467–470
55 Zhang, R. *et al.* (1995) Defective signalling through the T- and B-cell antigen receptors in lymphoid cells lacking the *vav* proto-oncogene. *Nature* 374, 470–473
56 Iritani, B.M. *et al.* (1999) Distinct signals mediate maturation and allelic exclusion in lymphocyte progenitors. *Immunity* 10, 713–722
57 Gärtner, F. *et al.* (1999) Immature thymocytes employ distinct signaling pathways for allelic exclusion versus differentiation and expansion. *Immunity* 10, 537–546
58 Michie, A.M. *et al.* (1999) Extracellular signal-regulated kinase (ERK) activation by the pre-T cell receptor in developing thymocytes *in vivo*. *J. Exp. Med.* 190, 1647–1655
59 Alberola-Ila, J. *et al.* (1998) Analysis of the role of MKK-4/Sek-1 in T cell development and apoptosis. *Int. Immunol.* 10, 1077–1082
60 Sabapathy, K. *et al.* (1999) JNK2 is required for efficient T-cell activation and apoptosis but not for normal lymphocyte development. *Curr. Biol.* 9, 116–125
61 Swat, W. *et al.* (1998) SEK1/MKK4 is required for maintenance of a normal peripheral lymphoid compartment but not for lymphocyte development. *Immunity* 8, 625–634
62 Barndt, R. *et al.* (1999) A novel role for HEB downstream or parallel to the pre-TCR signaling pathway during αβ thymopoiesis. *J. Immunol.* 163, 3331–3343
63 Winandy, S. *et al.* (1999) Pre-T cell receptor (TCR) and TCR-controlled checkpoints in T cell differentiation are set by Ikaros. *J. Exp. Med.* 190, 1039–1048
64 Miyazaki, T. (1997) Two distinct steps during thymocyte maturation from CD4$^-$CD8$^-$ to CD4$^+$CD8$^+$ distinguished in the early growth response (Egr)-1 transgenic mice with a recombinase-activating gene-deficient background. *J. Exp. Med.* 186, 877–885
65 Crabtree, G.R. (1999) Generic signals and specific outcomes: signaling through Ca^{2+}, calcineurin, and NF-AT. *Cell* 96, 611–614

66 Jiang, D. *et al.* (1996) **p53 prevents maturation to the CD4$^+$CD8$^+$ stage of thymocyte differentiation in the absence of T cell receptor rearrangement.** *J. Exp. Med.* 183, 1923–1928
67 Bogue, M.A. *et al.* (1996) **p53 is required for both radiation-induced differentiation and rescue of *V(D)J* rearrangement in scid mouse thymocytes.** *Genes Dev.* 10, 553–565
68 Maraskovsky, E. *et al.* (1997) **Bcl-2 can rescue T lymphocyte development in interleukin-7 receptor-deficient mice but not in mutant *rag-1*$^{-/-}$ mice.** *Cell* 89, 1011–1019
69 Newton, K. *et al.* (2000) **FADD/MORT1 regulates the pre-TCR checkpoint and can function as a tumour suppressor.** *EMBO J.* 19, 931–941
70 Henning, S.W. and Cantrell, D.A. (1998) **p56lck signals for regulating thymocyte development can be distinguished by their dependency on Rho function.** *J. Exp. Med.* 188, 931–939
71 Aifantis, I. *et al.* (1999) **Allelic exclusion of the T cell receptor β locus requires the SH2 domain-containing leukocyte protein (SLP)-76 adaptor protein.** *J. Exp. Med.* 190, 1093–1102
72 Yablonski, D. *et al.* (1998) **Uncoupling of nonreceptor tyrosine kinases from PLC-γ1 in an SLP-76-deficient T cell.** *Science* 281, 413–416
73 Williams, B.L. *et al.* (1999) **Phosphorylation of Tyr319 in ZAP-70 is required for T-cell antigen receptor-dependent phospholipase C-γ1 and Ras activation.** *EMBO J.* 18, 1832–1844
74 Sun, Z. *et al.* (2000) **PKC-θ is required for TCR-induced NF-κB activation in mature but not immature T lymphocytes.** *Nature* 404, 402–407
75 Levelt, C.N. *et al.* (1993) **Restoration of early thymocyte differentiation in T-cell receptor β-chain-deficient mutant mice by transmembrane signaling through CD3ε.** *Proc. Natl. Acad. Sci. U. S. A.* 90, 11401–11405
76 Levelt, C.N. *et al.* (1995) **Regulation of thymocyte development through CD3: functional dissociation between p56^{lck} and CD3ζ in early thymic selection.** *Immunity* 3, 215–222
77 Mombaerts, P. *et al.* (1992) **RAG-1 deficient mice have no mature B and T lymphocytes.** *Cell* 68, 869–877
78 Shinkai, Y. *et al.* (1992) **RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement.** *Cell* 68, 855–867
79 Jacobs, H. *et al.* (1994) **CD3 components at the surface of pro-T cells can mediate pre-T cell development *in vivo*.** *Eur. J. Immunol.* 24, 934–939
80 Shinkai, Y. and Alt, F.W. (1994) **CD3ε-mediated signals rescue the development of CD4$^+$CD8$^+$ thymocytes in RAG-2$^{-/-}$ mice in the absence of TCR β chain expression.** *Int. Immunol.* 6, 995–1001

letters

Do glucocorticoids participate in thymocyte development?

There is a substantial body of evidence suggesting that glucocorticoids affect T-cell development. In this issue, Godfrey and colleagues[1] review this literature in light of their recent finding that prenatal thymocyte development is grossly normal in glucocorticoid receptor knockout (GR$^{-/-}$) mice.

Some of the data in this area have been generated either in manipulated normal animals or in transgenic mice that express antisense GR transcripts in many tissues. Because of feedback regulation of systemic glucocorticoids on the hypothalamus–pituitary–adrenal (HPA) axis, and the fact that glucocorticoids are also produced locally in the thymus, it is difficult to know in these studies exactly how glucocorticoids or weak agonists/antagonists like RU-486 exert their effects. We have attempted to minimize the influence of adrenal steroids and the HPA axis by using two different experimental approaches: expression of GR antisense transcripts under the control of the *lck* proximal promoter – that is, only in thymocytes (TKO mice) – and fetal thymic organ culture (FTOC) in which glucocorticoid biosynthesis is inhibited. Both approaches have provided consistent data suggesting not only a role for glucocorticoids in T-cell development, but also possible molecular mechanisms. Do the data with the GR knockout mice now preclude a role for glucocorticoids in thymocyte development? We believe that at this time we do not have sufficient information to reach a conclusion.

We have proposed that glucocorticoids, by interfering with the nuclear consequences of TCR-mediated activation, set the signaling thresholds for different selection outcomes. This is supported by the finding that CD5 expression on double positive (DP) thymocytes, a sensitive indicator of TCR occupancy, is upregulated in an MHC-dependent manner when glucocorticoid signaling is attenuated *in vivo* or *in vitro*[2]. Antigen-specific selection has been addressed in several ways. Addition of metyrapone, an inhibitor of corticosterone synthesis, to FTOC caused the apoptosis of antigen-specific TCR transgenic DP thymocytes that normally undergo positive selection (reversed by corticosterone), but had no effect on viability if the MHC haplotype was incapable of presenting the selecting antigen[3]. In another approach, the number of T cells expressing TCRs that are positively selected (identified by the use of particular Vβ regions) was reduced by introduction of the GR antisense transgene[4]. Even more strikingly, TKO mice were found to have an altered TCR repertoire, being nonresponsive to pigeon cytochrome *c* but responding normally to other complex antigens[5]. Is selection altered in the GR$^{-/-}$ mice? Based on the available data it is not possible to say. Negative selection in response to potent stimuli appears to be grossly normal, but without dose–response curves it is not possible to say whether GR$^{-/-}$ DP thymocytes are unusually sensitive. Positive selection is even more problematic. Positive selection clearly occurs in GR$^{-/-}$ mice, but does the TCR repertoire differ from that of wild-type animals? At this time we do not know, but characterization of the effect of GR loss on MHC-dependent Vβ use in normal animals, and selection in antigen-specific αβ TCR transgenic mice, should shed light on this issue.

The major documented difference between thymuses from GR$^{-/-}$ and TKO mice or from normal mice cultured in FTOC with metyrapone is that only the former have normal numbers of DP cells. Is it possible to reconcile these very different findings? One possibility is that there might be strain-specific differences between the mice. We